REMARKS

Allowable Subject Matter

Applicants gratefully acknowledge the Examiner's indication that claims 1-36, 38-42, 46-61 and 71-82 are allowed.

Rejections of claims 68-70 Under 35 USC §112, first and second paragraphs

Both of these rejections relate to the recitation in the claims of inflammatory diseases. In the rejection under 35 USC §112, first paragraph, the Examiner alleges that the claims are non-enabled with respect to the treatment of inflammatory diseases in general. However, the claims do not recite merely treating inflammatory diseases, but instead recite treating inflammatory disease, resulting from decreased cyclic AMP levels, elevated phosphodiesterase 4 levels, or both. The rejection under 35 USC §112, second paragraph, appears to recognize that the claims do not recite merely treating inflammatory diseases, but the Examiner alleges that the scope is "unknown."

Contrary to the Examiner's comments, the use of PDE 4 inhibitors for treating inflammatory diseases resulting from decreased cyclic AMP levels and/or elevated phosphodiesterase 4 levels is extremely well know in the art.

At page 49, lines 13-18 of the specification, applicants list some prior disclosures concerning anti-inflammatory activity of PDE 4 inhibitors. See, for example, US 5,814,651 (assigned to Pfizer), already of record, states the following at column 1, lines 31-51:

> Since the recognition that cyclic AMP is an intracellular second messenger (E. W. Sutherland, and T. W. Rall, Pharmacol. Rev., 1960, 12, 265), inhibition of the phosphodiesterases have been a target for modulation and, accordingly, therapeutic intervention in a range of disease processes. More recently, distinct classes of PDE have been recognized (J. A. Beavo and D. H. Reifsnyder, TIPS, 1990, 11, 150), and their selective inhibition has led to improved drug therapy (C. D. Nicholson, R. A. Challiss and M. Shahid, TIPS, 1991, 12, 19). **More particularly, it has been recognized that inhibition of PDE type IV can lead to inhibition of inflammatory mediator release** (M. W. Verghese et al., J. Mol. Cell Cardiol., 1989, 12

(Suppl. II), S 61) **and airway smooth muscle relaxation** (T. J. Torphy in Directions for New Anti-Asthma Drugs, eds S. R. O'Donnell and C. G. A. Persson, 1988, 37, Birkhauser-Verlag). **Thus, compounds that inhibit PDE type IV, but which have poor activity against other PDE types, would inhibit the release of inflammatory mediators and relax airway smooth muscle** without causing cardiovascular effects or antiplatelet effects. (emphasis added)

Enclosed herewith is a copy of the Verghese et al. abstract mentioned above.

In addition, a word search using the USPTO patent database with the search terms "PDE IV" and "inflammation" produced no fewer than 279 hits (1976 to present). A similar search using "PDE IV" and "inflammatory" came up with 377 hits. The following are excerpts from just a sampling of the many US patents that recognize the anti-inflammatory activity of PDE 4 inhibitors and their mechanism of treating inflammations.

US 6,043,263 (assigned to Byk Gulden): column 14, lines 33-50

The compounds according to the invention have valuable pharmacological properties which make them commercially utilizable. **As selective cyclic nucleotide phosphodiesterase (PDE) inhibitors (namely of type IV), they are suitable** on the one hand as bronchial therapeutics (for the treatment of airway obstructions on account of their dilating but also on account of their respiratory rate- or respiratory drive-increasing action) and for the elimination of erectile dysfunction on account of the vasodilatory action, but on the other hand **especially for the treatment of disorders, in particular of inflammatory nature, e.g. of the airways (asthma prophylaxis), of the skin, of the intestine, of the eyes and of the joints, which are mediated by mediators such as histamine, PAF (platelet-activating factor), arachidonic acid derivatives such as leukotrienes and prostaglandins, cytokines, interleukins, chemokines, alpha-, beta- and gamma-interferon, tumor necrosis factor (TNF) or**

oxygen radicals and proteases. (emphasis added)

US 6,294,564 (assigned to Byk Gulden): column 18, line 38-coluim 19, line 13

On account of their PDE-inhibiting properties, the compounds according to the invention can be employed in human and veterinary medicine and therapeutics, where they can be used, for example, **for the treatment and prophylaxis of the following illnesses: acute and chronic (in particular inflammatory and allergen-induced) airway disorders of various origins** (bronchitis, allergic bronchitis, bronchial asthma, emphysema, COPD); **dermatoses (especially of proliferative, inflammatory and allergic type)** such as, for example, psoriasis (vulgaris), toxic and allergic contact eczema, atopic eczema, seborrhoeic eczema, lichen simplex, sunburn, pruritus in the anogenital area, alopecia areata, hypertrophic scars, discoid lupus erythematosus, follicular and wide-area pyodermias, endogenous and exogenous acne, acne rosacea and other proliferative, **inflammatory and allergic skin disorders;** disorders which are based on an excessive release of TNF and leukotrienes, e.g. disorders of the arthritis type (rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis and other arthritic conditions), disorders of the immune system (AIDS, multiple sclerosis), graft-versus-host reactions, transplant rejection reactions, symptoms of shock [septic shock, endotoxin shock, gram-negative sepsis, toxic shock syndrome and ARDS (adult respiratory distress syndrome)], and **generalized inflammations in the gastrointestinal area** (Crohn's disease and ulcerative colitis); disorders which are based on allergic and/or chronic, faulty immunological reactions in the area of the upper airways (pharynx, nose) and the adjacent regions (paranasal sinuses, eyes), such as, for example, allergic rhinitis/sinusitis, chronic rhinitis/sinusitis, allergic conjunctivitis and nasal polyps; but also disorders of the heart which can be treated by PDE inhibitors, such as, for example, cardiac insufficiency, or disorders which can be treated on account of the tissue-relaxant action of the PDE inhibitors, such as, for

example, erectile dysfunction or colics of the kidneys and the ureters in connection with kidney stones. In addition, the compounds according to the invention can be employed for the treatment of diabetes insipidus and disorders in connection with disturbances of brain metabolism, such as, for example, cerebral senility, senile dementia (Alzheimer's dementia), multiinfarct dementia or alternatively disorders of the CNS, such as, for example, depressions or arteriosclerotic dementia. (emphasis added)

US 6,294,564 (assigned to Byk Gulden): column 20, line 41-coluim 21, line 3

Substances which inhibit chemoluminescence and cytokine secretion and the secretion of inflammatory mediators on inflammatory cells, in particular neutrophilic and eosinophilic granulocytes, T lymphocytes, monocytes and macrophages, are those which inhibit PDE4. This isoenzyme of the phosphodiesterase families is particularly represented in granulocytes. Its inhibition leads to an increase in the intracellular cyclic AMP concentration and thus to the inhibition of cell activation. **PDE4 inhibition by the substances according to the invention is thus a central indicator of the suppression of inflammatory processes** (Giembycz MA, Could isoenzyme-selective phosphodiesterase inhibitors render bronchodilatory therapy redundant in the treatment of bronchial asthma?. Biochem Pharmacol 1992, 43, 2041-2051; Torphy TJ et al., Phosphodiesterase inhibitors: new opportunities for treatment of asthma. Thorax 1991, 46, 512-523; Schudt C et al., Zardaverine: a cyclic AMP PDE 3/4 inhibitor. In "New Drugs for Asthma Therapy", 379-402, Birkhauser Verlag Basle 1991; Schudt C et al., Influence of selective phosphodiesterase inhibitors on human neutrophil functions and levels of cAMP and Ca; Naunyn-Schmiedebergs Arch Pharmacol 1991, 344, 682-690; Tenor H and Schudt C, Analysis of PDE isoenzyme profiles in cells and tissues by pharmacological methods. In "Phosphodiesterase Inhibitors", 21-40, "The Handbook of Immunopharmacology", Academic Press, 1996; Hatzelmann A et al., Enzymatic and functional aspects of

dual-selective PDE3/4-inhibitors. In "Phosphodiesterase Inhibitors", 147-160. "The Handbook of Immunopharmacology", Academic Press, 1996).

US 6,555,572 (assigned to Inflazyme Pharmaceuticals): column 2, lines 21-40

For additional and more detailed discussion of PDE enzymes, including the history of their discovery, their characterization and classification, their in vivo activity, their inhibition by small organic molecules, and current clinical efforts directed to providing pharmaceutical compositions containing these small molecules, see, e.g., Bumouf, C. et al. "Phosphodiesterase 4 Inhibitors" Annual Reports in Medicinal Chemistry, Vol. 33, Chap. 10, pp 91-109, 1998 (Bristol, J. A., ed.); Essayan, D. M. "Cyclic Nucleotide Phosphodiesterase (PDE) Inhibitors and Immunomodulation" Biochemical Pharmacology 57:965-973, 1999; Souness, J. E. and Foster, M. "Potential of phospodiesterase type IV inhibitors in the treatment of rheumatoid arthritis" Idrugs 1(5):541-553, 1998; **Souness, J. E. et al. "Immunosuppressive and anti-inflammatory effect of cAMP phosphodiesterase (PDE) type 4 inhibitors" Immunopharmacology 47: 127-162, 2000**; and Torphy, T. J. "Phosphodiesterase Isozymes" Am J. Respir. Crit. Care Med. 157:351-370, 1998, as well as the numerous references cited in these articles. (emphasis added)

US 6,204,275 (assigned to Merck Frost Canada & Co.) column 1, lines 34-48

The availability of PDE isotype selective inhibitors has enabled the role of PDEs in a variety of cell types to be investigated. **In particular it has been established that PDE IV controls the breakdown of cAMP in many inflammatory cells**, for example, basophils (Peachell P. T. et al., (1992) J. Immunol., 148: 2503-2510) and eosinophils (Dent G. et al., (1991) Br. J. Pharmacol., 103: 1339-1346) **and that inhibition of this isotype is associated with the inhibition of cell activation.**

Furthermore, elevation of cAMP in airway smooth muscle has a spasmolytic effect. Consequently PDE IV inhibitors are currently being developed as potential anti-inflammatory drugs particularly for the prophylaxis and treatment of asthma, by achieving both anti-inflammatory and bronchodilator effects. (emphasis added)

US 5,889,014 (assigned to Euro-Celtique) column 1, line 54- column 2, line 8

The structure-activity relationships (SAR) of isozyme-selective inhibitors has been discussed in detail, e.g., in the article of Theodore J. Torphy, et al., "Novel Phosphodiesterase Inhibitors For The Therapy Of Asthma", Drug News & Prospectives, 6(4) May 1993, pages 203-214. The PDE enzymes can be grouped into five families according to their specificity toward hydrolysis of cAMP or cGMP, their sensitivity to regulation by calcium, calmodulin or cGMP, and their selective inhibition by various compounds. PDE I is stimulated by Ca.sup.2+ /calmodulin. PDE II is cGMP-stimulated, and is found in the heart and adrenals. PDE III is cGMP-inhibited, and inhibition of this enzyme creates positive inotropic activity. **PDE IV is cAMP specific, and its inhibition causes airway relaxation, anti-inflammatory and ant-depressant activity.** PDE V appears to be important in regulating cGMP content in vascular smooth muscle, and therefore PDE V inhibitors may have cardiovascular activity. (emphasis added)

US 6,258,833 (assigned to ICOS Corporation): column 1, lines 6-17, and column 3, lines 44-57

The present invention relates to a series of compounds that are potent and selective inhibitors of cyclic adenosine 3',5'-monophosphate specific phosphodiesterase (cAMP specific PDE). **In particular, the present invention relates to a series of novel pyrrolidine compounds which are useful for inhibiting the function of cAMP specific PDE, in particular, PDE4, as well as methods of making the same, pharmaceutical**

compositions containing the same, and their use as therapeutic agents, for example, in treating inflammatory diseases and other diseases involving elevated levels of cytokines and proinflammatory mediators. (emphasis added)

Investigators have shown considerable interest in the use of PDE4 inhibitors as anti-inflammatory agents. **Early evidence indicates that PDE4 inhibition has beneficial effects on a variety of inflammatory cells such as monocytes, macrophages, T-cells of the Th-1 lineage, and granulocytes. The synthesis and/or release of many proinflammatory mediators, such as cytokines, lipid mediators, superoxide, and biogenic amines, such as histamine, have been attenuated in these cells by the action of PDE4 inhibitors.** The PDE4 inhibitors also affect other cellular functions including T-cell proliferation, granulocyte transmigration in response to chemotoxic substances, and integrity of endothelial cell junctions within the vasculature. (emphasis added)

US 5,124,455 (assigned to American Home Products): column 1, lines 32-55

Cyclic AMP concentrations within the living cell are determined by both the rate of its synthesis by adenylate cyclase and the rate of its degradation by phosphodiesterases (PDEs). Thus, either stimulating adenylate cyclase or inhibiting PDEs in pulmonary tissues can result in anti-asthmatic activities. **This invention relates to compounds that inhibit a specific PDE, often called PDE IV, which selectively metabolizes cAMP** and which is insensitive to the modulatory effects of guanosine cyclic 3':5' monophosphate (cGMP) and calcium. This PDE is found in both respiratory smooth muscle and inflammatory cells, and has been demonstrated to be a principal regulator of cAMP in these tissues [see Torphy and Cieslinski, Molecular Pharmacology, 37, 206 (1990), and Dent et al., British Journal of Pharmacology, 90, 163P (1990)].

> **Consequently, the compounds of the invention are bronchodilatory and antiinflamatory, and exhibit activity in animal models of allergic and nonallergic asthma.** However, because the compounds of the invention have not been found to inhibit other forms of PDE, they are deemed to be more selective and safer anti-asthmatics than nonselective PDE inhibitors currently used for the treatment of asthma, such as theophylline.

The assertions presented in the rejection present no reason to doubt that PDE4 inhibitors can be used for the treatment of inflammatory disease resulting from decreased cyclic AMP levels and/or elevated phosphodiesterase 4 levels, especially since one of ordinary skill in the art is well aware of use of PDE4 inhibitors for just such treatments.

All that is required under the statute is objective enablement. It is not required that applicants' disclosure present specific test results. See, e.g., *In re Marzocchi et al.*, 169 USPQ 367, 369(CCPA 1971):

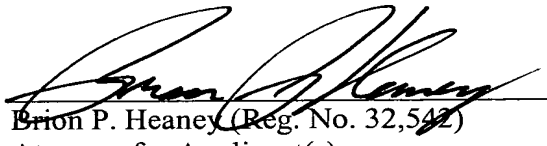
> The first paragraph of §112 requires nothing more than objective enablement. How such a teaching is set forth, either by the use of illustrative examples or by broad terminology, is of no importance.

An application disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken in compliance with the enabling requirement of the first paragraph 35 U.S.C. § 112 unless there is reason to doubt the objective truth of statements contained therein relied on for enabling support. *In re Brana, 51 F.3d 1560,* 34 USPQ2d 1436 (Fed. Cir. 1995). *Fiers v. Revel, 984 F.2d 1164,* 24 USPQ2d 1601 (Fed. Cir. 1993). Furthermore, as stated in *In re Marzocchi*, 169 U.S.P.Q. 367, 369 (CCPA 1971), the PTO must have adequate support for its challenge to the credibility of applicant's statements of utility. See also *In re Bundy*, 209 USPQ 48 (CPA 1981).

In view of the above remarks, it is respectfully submitted that Applicants'

disclosure provides more than sufficient guidance to objectively enable one of ordinary skill in the art to make and use the claimed invention with no more than routine experimentation. The rejection does not present sufficient reasons to doubt the veracity of the enabling statements set forth in the disclosure. Thus, the rejection under 35 USC §112, first paragraph, should be withdrawn. Furthermore, the language of the claims is sufficient for one of ordinary skill in the art, being well aware of the anti-inflammatory activities of PDE 4 inhibitors, can readily understand the scope of the present claims. Withdrawal of the rejections under 35 U.S.C. §112, first and second paragraph, is respectfully requested.

Respectfully submitted,



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181 **ANTI-NEUTROPHIL ACTIVITY OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE INHIBITORS WITH VARYING CARDIOTONIC POTENCIES.** M.W. Verghese, T.A. Brown, O. Irsula, T.G. Ropchak, and C. Frangakis. Glaxo Laboratories, Research Triangle Park, N.C. 27709

Since cAMP-elevating drugs inhibit superoxide (O_2^-) production of human neutrophils (PMN) in response to the chemotactic peptide fMet-Leu-Phe (FMLP), we compared the effects of inotropic cyclic nucleotide phosphodiesterase (PDE) inhibitors on this response. The non-specific PDE inhibitors papaverine, isobutylmethylxanthine (IBMX) and theophylline had IC_{50} values of ca. 10, 250 and >500 uM for FMLP-induced O_2^- production, resp.. Of the selective PDE inhibitors, RO201274 was comparable to papaverine, while milrinone, amrinone or zaprinast were similar to IBMX, and isomazole or enoximone were slightly better than theophylline in decreasing O_2^- production. Ca^{2+}-calmodulin (CaM) dependent cGMP- or cAMP-PDE (Type I) activity was undetectable in sonicated PMNs. In these sonicates, the most potent inhibitors of the high affinity cAMP-PDE (Type IV) activity were papaverine and RO201274, followed by IBMX and milrinone, while the potencies for the low affinity cAMP-PDE was RO201274 > IBMX > papaverine. In contrast, the inhibitory potencies against the CaM independent cGMP-PDE (Type II) in sonicated PMN were papaverine > IBMX > isomazole, zaprinast > milrinone, RO201274, amrinone. Of these, only zaprinast and isomazole were clearly specific for cGMP-PDE. The correlation coefficients between inhibition of O_2^- production in intact PMN and PDE-activity in sonicates were 0.80, 0.61, and 0.45 for high and low affinity cAMP-PDE or cGMP-PDE, resp.. Thus, drugs that specifically affect the cGMP-insensitive Type IV PDE (e.g. RO201274) are more effective inhibitors of PMN O_2^- production than cardiotonic drugs with selectivity for the cGMP inhibitable Type IV PDE (e.g. milrinone).

182 SITE-SPECIFIC MUTAGENESIS OF PHOSPHOLAMBAN. STUDIES OF RESIDUES INVOLVED IN PENTAMER FORMATION AND PHOSPHORYLATION. J. Fujii, K. Maruyama, *M. Tada, and D. H. MacLennan. Banting and Best Department of Medical Research, Charles H. Best Institute, University of Toronto, Toronto, Ontario M5G 1L6, Canada and *First Department of Medicine and Department of Pathophysiology, Osaka University School of Medicine, Osaka 553, Japan

The cDNA encoding phospholamban, a regulatory protein of sarcoplasmic reticulum Ca^{2+}-ATPase, has been expressed in COS-1 cells. The expressd protein formed a pentamer and was phosphorylated by protein kinases. Although several amino acid were substituted by site-specific mutagenesis, only mutations of intramembranous cysteine residues at positions 36, 41, and 46 diminished the stability of the pentameric form, suggesting that these residues might be responsible for the intermolecular interactions which prevent SDS from binding to the hydrophobic portion of the pentamer. We have also confirmed that Ser^{16} and Thr^{17} are the residues uniquely phosphorylated by cAMP- and calmodulin-dependent protein kinases, respectively. In addition to these residues, Arg^{13}-Arg^{14} are necessary for phosphorylation by both kinases and Arg^{9} is important for calmodulin- but not for cAMP-dependent phosphorylation.

183 **STRUCTURAL MODEL OF OLIGOMERIC ASSEMBLY OF PHOSPHOLAMBAN.** Y.Kimura, M.Kadoma, M.Inui, Y.Kijima, M.Tada, J.Fujii*, D.H.MacLennan*, and Y.Oyama**. Osaka University School of Medicine, Osaka, Japan, *Charles H. Best Institute, University of Toronto, Toronto, Canada, and **Suntory Institute for Biomedical Research, Osaka, Japan.

Phospholamban, a putative regulator of the Ca-ATPase of cardiac sarcoplasmic reticulum, consists of five monomers (MW=6,080), forming a homo-oligomer (Mr≈27K). The complete amino acid sequence of the monomer was recently deduced from cDNA cloning. From secondary structure prediction and circular dichroism spectrum analysis, the phospholamban monomer is enriched in α-helix. The Cys residues in the C-terminal hydrophobic helix, domain II, seem to be responsible for oligomeric assembly, since substitution of one of the three Cys residues in domain II (Cys 36, 41, and 46), especially Cys 41, make the oligomer unstable. These three Cys residues are placed at about 120° to each other around the axis of the α-helix. The inter-molecular interaction, forming a cluster of domain II α-helices, is not due to disulfide bonds but to non-covalent reactions in the hydrophobic environment, presumably established between side chains of Cys 36 and Cys 41. Taking this information into account, we present the most suitable structural model of the phospholamban oligomer using computer simulation analyses. In this model, the structure of domain II is a "left-handed supercoil" with each α-helix tilted at 20°, which makes Cys 41 interact non-covalently, with Cys 36 of the neighboring monomer in an extremely hydrophobic milieu in the membrane interior.